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Monitoring of the activity and redox state of mitochondrial complexes in physiological and pathological conditions using in-gel kinetics acquisition and integrating sphere spectroscopy

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Recent evidence suggests that post-translational modifications may modify the enzymatic activity of mitochondrial oxidative phosphorylation complexes (MOPCs). Herein we describe the modification of native gel assays and optical spectroscopy techniques to monitor MOPCs activity in order to take advantage of the unique properties of these approaches. In-gel enzymatic assays are performed by using reaction-dependent deposition of precipitates quantified at fixed time points, limiting the analysis of reaction kinetics. To monitor reaction kinetics, we developed an imaging system that continuously collects in-gel enzymatic activity in turbid reaction media. Cardiac Complex V in-gel kinetics were non-linear with a significant lag phase followed by two linear phases. Taking samples from arrested (low work) and dobutamine treated (high work) hearts revealed alterations in Complex V activity primarily due to changes in the lag phase. The nature of the Complex V initial lag was unexpected and still under investigation. The native gel approach is limited to only partial MOPC reactions with no membrane potential and altered protein conformations. To overcome these limitations, we developed a rapid scanning spectrometer coupled to a center mounted integrating sphere to minimize scattering effects for monitoring MOPCs activity under normal redox and substrate conditions. Individual chromophore spectra were used to accurately fit the entire redox sensitive mitochondrial optical spectrum to quantify MOPC redox states. In standard state 4 to 3 transitions, the major redox crossovers were found between cytochrome c_1 and b_H in Complex III, while a novel crossover was detected within Complex IV between the Ferryl and Peroxy intermediate states, based on the optical spectra. These data suggested a novel dependence of reducing equivalent residence within Complex IV with alteration in flow. The MOPC redox state, oxygen consumption and membrane potential were used to calculate the effective conductance of the entire chain as well as individual MOPCs. MOPC activity was evaluated in mitochondria from control and ischemia-reperfusion using both methods. Surprisingly, a systemic down-regulation of all MOPC activity was detected despite maintenance of driving force that was associated with a 33% decrease in maximum ATP production rate. These data imply a coordinated diminution of MOPC activity in this pathological state and not one particular MOPC as a target of reperfusion injury.

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Sirt3 affects the function and stability of OXPHOS complexes in response to metabolic challenges

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While the basic principles of regulation of mitochondrial function are established, understanding of the adaptation of organellar function in response to cellular stress is just emerging. A possible mode of regulation includes posttranslational modifications of key components involved in mitochondrial function. Acetylation of mitochondrial proteins involved mitochondrial metabolism that has been described. Mitochondria possess a set of NAD^+-dependent deacetylase, the mitochondrial sirtuins. One member if this family, namely Sirt3, targets several enzymes of mitochondrial metabolism and biogenesis. Sirt3 expression is induced under several stress conditions, which makes it a prime candidate to mediate adaptation of mitochondrial processes to stress. We have addressed this question by analyzing the role of Sirt3 in regulation of OXPHOS function and mitochondrial biogenesis under cellular stress. We subjected Sirt3 deficient, Sirt3 over-expressing and control cells to different kinds of nutrient starvation and forced respiration. Our data show that the absence of Sirt3 causes a defect in mitochondrial ATP generating capacity which is largely aggrevated during cellular stress. Use of a catalytic inactive variant of Sirt3 clearly demonstrates that this lack of metabolic flexibility is linked to the deacetylase activity of Sirt3.

Recently it was reported that the mitochondrial protein synthesis machinery, that is responsible for the generation of mtDNA encoded proteins, is a target of Sirt3. To get a deeper insight if and how Sirt3 might influence mitochondrial ATP synthesis through modulation of mitochondrial protein synthesis, we analyzed mitochondrial translation in Sirt3 deficient, Sirt3 over-expressing and control cells subjected to different kinds of nutrient starvation and forced respiration. We found that Sirt3 is required for adaptation of this process in the analyzed stress conditions. Interestingly we found that Sirt3 affects the turnover and stability of newly-synthesized proteins as well as of assembled OXPHOS complexes and supercomplexes.

Altogether our data show that Sirt3 is an important regulator of mitochondrial ATP synthesis and that part of this regulatory pathway involves augmenting mitochondrial protein synthesis and protein turnover. Hence our data contribute to a further understanding of the molecular significance of Sirt3 in controlling mitochondrial metabolism and highlight the importance of stress-regulated mitochondrial function.

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Energy metabolism of endothelial cells, influence of TNF-alpha

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TNF-alpha is one of pivotal mediators of inflammatory response, hence, prosurvival pathway in endothelial cells is probably due to activation of NFkB. It also stimulates proapoptotic events including an excessive mitochondrial ROS production. Here a mitochondrial response in human endothelial cells (EA.hy926) stimulated with TNF-alpha was investigated. TNF-alpha stimulated ROS generation and increased ICAM protein level — two changes enumerate as a inflammation factors. These results paralleled with increased oxygen consumption as well as level of MnSOD and UCP2 protein content. They also correlated with a rise of transcriptional factors like TFAM, NRF1 and PGC1-alpha which are involved in regulation of mitochondrial biogenesis. Moreover, elevated level of selected respiratory chain proteins as